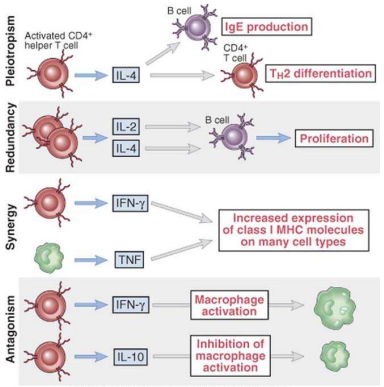
LEZIONE 5

Le citochine

Proprietà delle citochine



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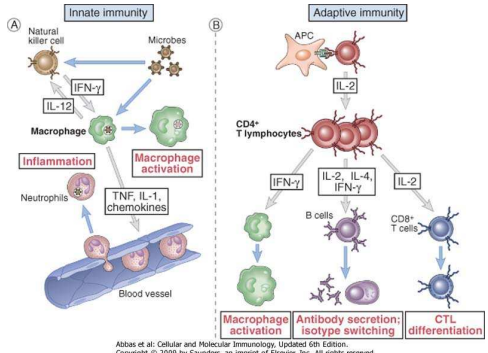
Proprietà delle citochine (cont.)

- Secrezione limitata e di breve durata
- Azioni locali o sistemiche (autocrine, paracrine, endocrine)
- Legano recettori con alta affinità
- Regolazione dei livelli di espressione da parte di fattori esterni (priming)
- Conseguenza dell'azione delle citochine: espressione genica, proliferazione o morte cellulare (a volte)
- Stretta regolazione dell'azione delle citochine (recettori decoy; inibitori di chinasi; attivazione fosfatasi; blocco dei fattori trascrizionali)



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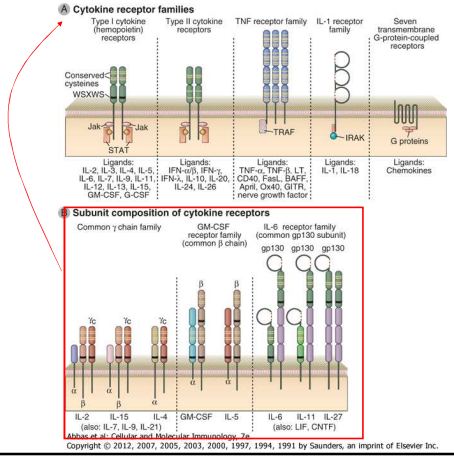
C: Haematopoietic cytokines D: Chemokines



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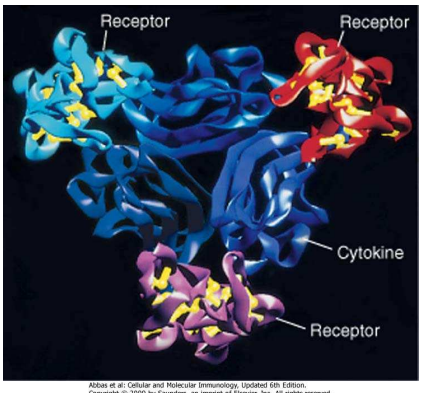
IL Tumor Necrosis Factor (TNF-α)



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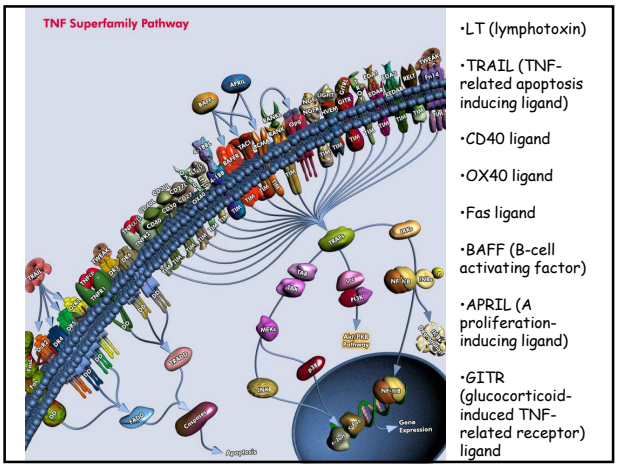
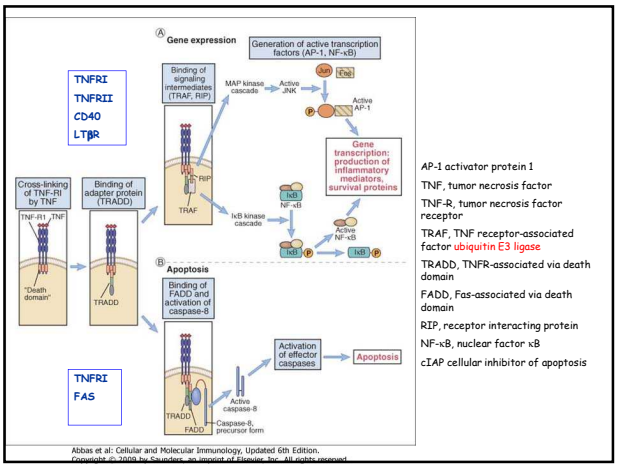


- LT (lymphotoxin)
- TRAIL (TNF-related apoptosis inducing ligand)
- CD40 ligand
- OX40 ligand
- Fas ligand
- BAFF (B-cell activating factor)
- APRIL (A proliferation-inducing ligand)
- GITR (glucocorticoid-induced TNF-related receptor) ligand



AP-1 activator protein 1
TNF, tumor necrosis factor
TNF-R, tumor necrosis factor receptor
TRAF, TNF receptor-associated factor ubiquitin E3 ligase
TRADD, TNFR-associated via death domain
FADD, Fas-associated via death domain
RIP, receptor interacting protein
NF-κB, nuclear factor κB
cIAP cellular inhibitor of apoptosis

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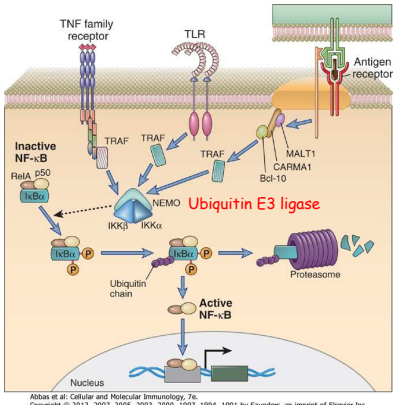
Attivazione canonica di NFκB



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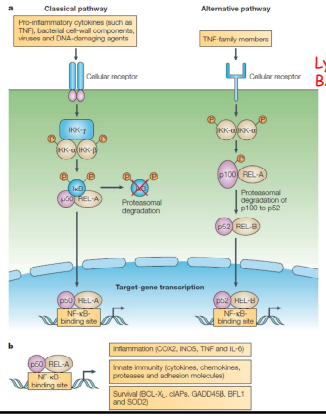
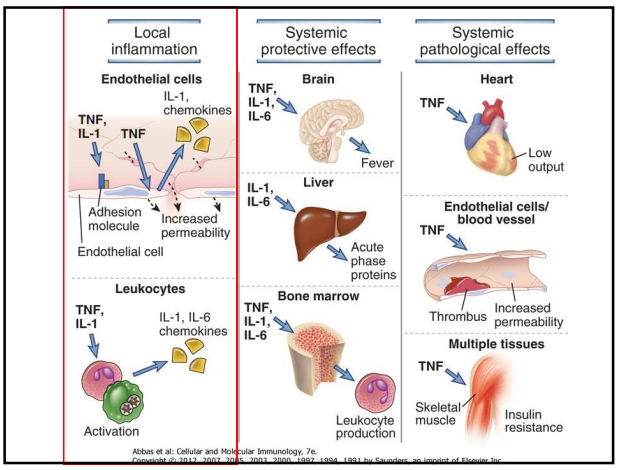
Attivazione canonica e non canonica di NFκB



Lymphotoxin β receptor
BAFF receptor



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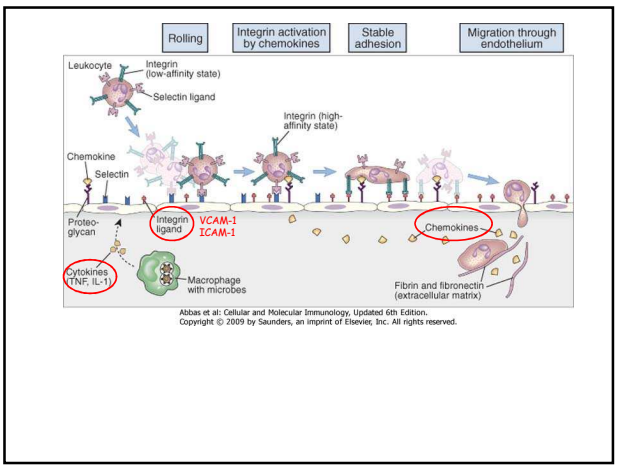

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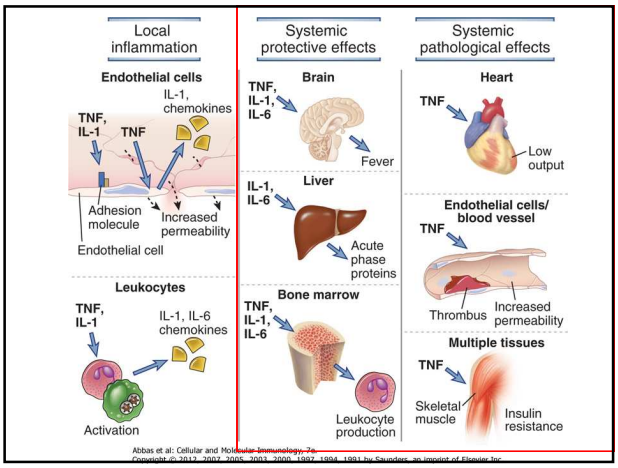

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Risposta della fase acuta

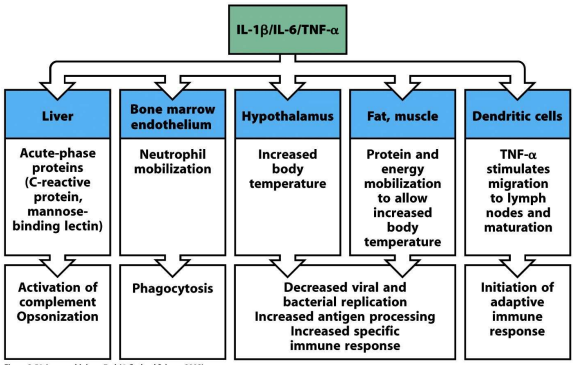


Figure 2-51 Immunobiology, 7ed. (© Garland Science 2008)

Effetti dannosi del TNF

- Febbre
- Cachessia (danno muscolare)
- Shock (diminuzione contrattilità miocardica e tono vascolatura)
- Trombosi > fattore tissutale e < trombomodulina (esito: DIC)
- Ipoglicemia

↓

Shock settico

Proteine sieriche della risposta della fase acuta

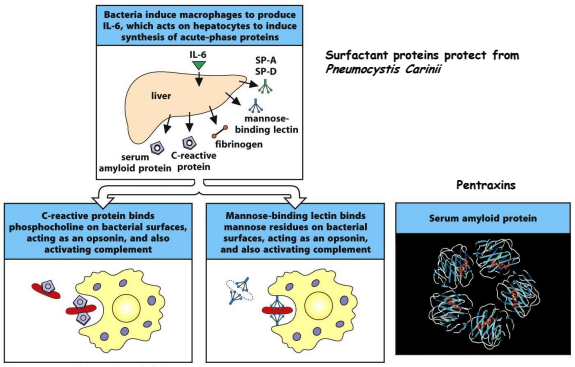


Figure 2-52 Immunobiology, 7ed. (© Garland Science 2008)

Attivazione macrofagi: un ponte tra immunità innata ed adattativa

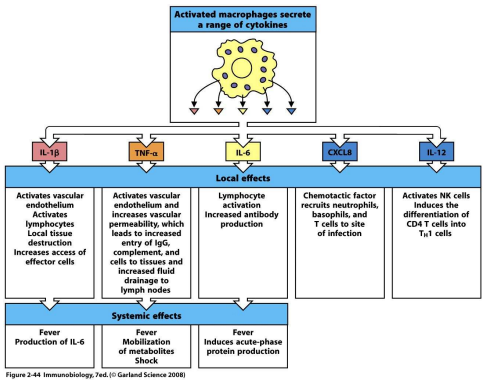
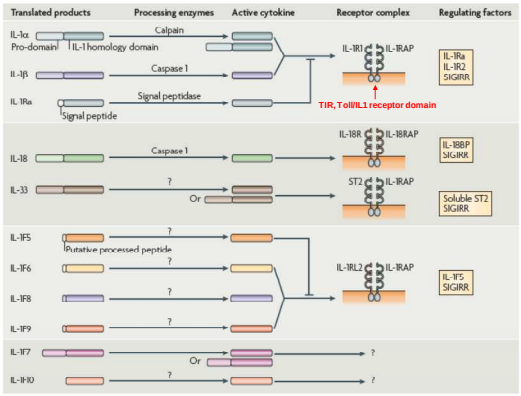


Figure 2-44 Immunobiology, 7ed. (© Garland Science 2008)

La famiglia dell'interleuchina 1



Caspasi 1 oppure ICE, interleukin 1 converting enzyme

Table 1. New Approved Designations for the Human NLR Family Members

NLR Family HGNC-Approved Symbol	Approved Name	Other Names and Aliases	Domain Organization	Protein Sequence
NLRA	CIITA	class II, major histocompatibility complex, transactivator	NLRA; MHC2TA; C2TA	(CARD)-AD-NACHT-NAD-LRR	NP_000237
NLRB	NAIP	NLR family, apoptosis inhibitory protein	NLRB1; BIRC1; CLR5.1	BIRs-NACHT-LRR	NP_004527
NLRC	NOD1	nucleotide-binding oligomerization domain containing 1	NLRC1; CARD4; CLR7.1	CARD-NACHT-NAD-LRR	NP_006083
NLRC	NOD2	nucleotide-binding oligomerization domain containing 2	NLRC2; CARD15; CD; BLAU; IBD1; PSORAS1; CLR16.2	CARD2s-NACHT-NAD-LRR	NP_071445
NLRC	NLRC3	NLR family, CARD domain containing 3	NOD3; CLR16.2	CARD-NACHT-NAD-LRR	NP_849172
NLRC	NLRC4	NLR family, CARD domain containing 4	CARD12; CLAN; CLR2.1; IPAF	CARD-NACHT-NAD-LRR	NP_067032
NLRC	NLRC5	NLR family, CARD domain containing 5	NOD27; CLR16.1	CARD-NACHT-NAD-LRR	NP_115582
NLRP	NLRP1	NLR family, pyrin domain containing 1	NALP1; DEFCAP; NAC; CARD7; CLR17.1	PYD-NACHT-NAD-LRR-FIIND-CARD	NP_127497
NLRP	NLRP2	NLR family, pyrin domain containing 2	NALP2; PYPAF2; NBS1; PAN1; CLR19.9	PYD-NACHT-NAD-LRR	NP_060322
NLRP	NLRP3	NLR family, pyrin domain containing 3	CIAS1; PYPAF1; Cryopyrin; CLR1.1; NALP3	PYD-NACHT-NAD-LRR	NP_004896
NLRP	NLRP4	NLR family, pyrin domain containing 4	NALP4; PYPAF4; PAN2; RNR2; CLR19.5	PYD-NACHT-NAD-LRR	NP_604393
NLRP	NLRP5	NLR family, pyrin domain containing 5	NALP5; PYPAF8; MATER; PAN11; CLR19.6	PYD-NACHT-NAD-LRR	NP_703148
NLRP	NLRP6	NLR family, pyrin domain containing 6	NALP6; PYPAF5; PAN3; CLR11.4	PYD-NACHT-NAD-LRR	NP_612202
NLRP	NLRP7	NLR family, pyrin domain containing 7	NALP7; PYPAF3; NOD12; PAN7; CLR19.4	PYD-NACHT-NAD-LRR	NP_996611
NLRP	NLRP8	NLR family, pyrin domain containing 8	NALP8; PAN4; NOD16; CLR19.2	PYD-NACHT-NAD-LRR	NP_789781
NLRP	NLRP9	NLR family, pyrin domain containing 9	NALP9; NOD6; PAN17; CLR19.1	PYD-NACHT-NAD-LRR	NP_789790
NLRP	NLRP10	NLR family, pyrin domain containing 10	NALP10; PAN5; NOD9; PYNOD; CLR11.1	PYD-NACHT-NAD	NP_789791
NLRP	NLRP11	NLR family, pyrin domain containing 11	NALP11; PYPAF6; NOD17; PAN10; CLR19.4	PYD-NACHT-NAD-LRR	NP_659444
NLRP	NLRP12	NLR family, pyrin domain containing 12	NALP12; PYPAF7; Monarch1; RNO9; PAN6; CLR19.3	PYD-NACHT-NAD-LRR	NP_653288
NLRP	NLRP13	NLR family, pyrin domain containing 13	NALP13; NOD14; PAN13; CLR19.7	PYD-NACHT-NAD-LRR	NP_789790
NLRP	NLRP14	NLR family, pyrin domain containing 14	NALP14; NOD5; PAN8; CLR11.3	PYD-NACHT-NAD-LRR	NP_789790
NLRX	NLRX1	NLR family member X1	NOD9; CLR11.2	X-NACHT-NAD-LRR	NP_078894

The following abbreviations are used: AD, acidic activation domain; CARD, caspase activating and recruitment domain; LRR, leucine-rich repeat; NACHT, domain present in NAIP, CIITA, HET-E, and TP-1; BIR, baculovirus inhibitor of apoptosis repeat; PYD, pyrin domain; and NAD, NACHT associated domain.

NLR, (NOD)-like receptors

NOD, nucleotide-binding oligomerization domain

NLR, NACHT-LRR receptors

NLR, nucleotide-binding domain and leucine-rich repeat containing gene family

LRR, carboxy-terminal leucine-rich repeats (s)

PYD, pyrin domain

CARD, caspase-recruitment domain

BIR, baculovirus inhibitor of apoptosis protein repeat

NLRP3, NLR family, pyrin domain-containing 3

ASC, apoptosis-associated speck-like protein containing CARD

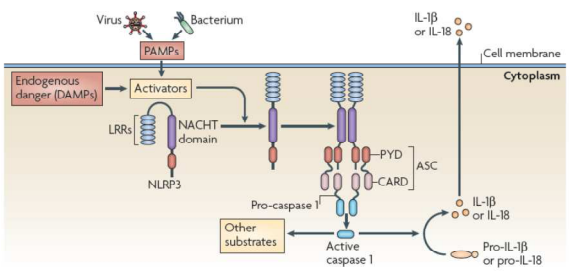
NACHT, domain present in NAIP, CIITA, HET-E and TP-1

Three main subfamilies according to the effector domain:

- PYD
- CARD
- BIR

Inflammasoma: sensore di DAMP e di PAMP

NLRP3, NLR family, pyrin domain-containing 3

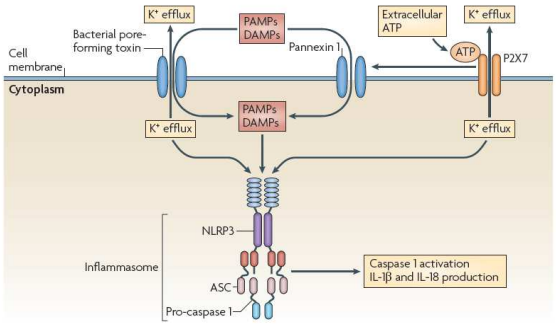


Attenzione: la pro-IL-1 deve essere sintetizzata *ex novo* dopo stimolo dei TLR (ed attivazione di NF-κB) mentre pro-IL-18 e pro-IL-33 sono costitutivamente espresse nei tessuti epiteliali e cellule di derivazione dal midollo. IL-33 non è attivata dall'inflammasoma

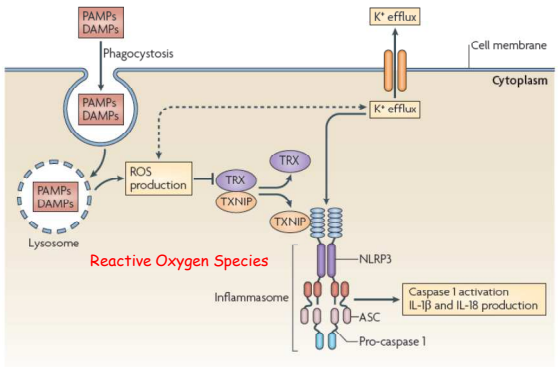
Table 1 | Proposed signalling pathways for NLRP3 inflammasome activators

Activator	Proposed signalling pathway	Refs
Microorganisms (PAMPs)		
Sendai virus	ND	31
Influenza virus	ND	31,32
Adenovirus	ROS	*
Candida albicans	ROS	33
Saccharomyces cerevisiae	ROS	*
Staphylococcus aureus	ROS	10
Listeria monocytogenes	ND	10,34
Bacterial pore-forming toxins	ROS	*
Endogenous danger signals (DAMPs)		
Extracellular ATP	ROS and channel formation	10
Hyaluronan	ND	35
Glucose	ROS	24
MSU	ROS	12
Amyloid-β	Lysosome rupture	21
Environmental irritants		
Skin irritants	ROS	*
Imidazoquinoline compounds	ROS	*
Silica	ROS and lysosome rupture	22,36
Asbestos	ROS	25,36
Alum	ROS and lysosome rupture	37

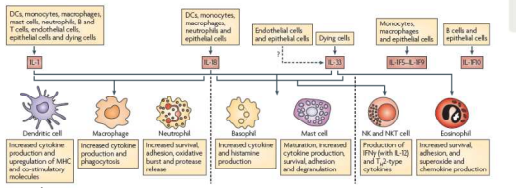
DAMPs, damage-associated molecular patterns; MSU, monosodium urate; ND, not determined; NLRP3, NLR family, pyrin domain-containing 3; PAMPs, pathogen-associated molecular patterns; ROS, reactive oxygen species. *C. Dostert and J.T., unpublished observations.



Modello unificatore: produzione di ROS ed attivazione inflammasoma



TXNIP, thioredoxin-interacting protein; TRX, thioredoxin



Azioni sul sistema immunitario innato

- IL-1α, IL-1β ed IL-18 contribuiscono alla risposta al danno tissutale acuto (attivazione endotelio e richiamo dei neutrofili, es. infarto)
- IL-1β è dotato di effetti sistemici simili ma non uguali al TNF
- IL-33 favorisce degranulazione mastociti, attivazione eosinofili con azione antiparassitaria, produzione di IL-4 e IL-13
- IL-1, IL-18 e IL-33 influenzano la produzione di IL-4 ed IL-13 dai basofili
- IL-18 e IL-33 attivano le NK a produrre IFN e IL-12 ed essere più citotossiche

Azioni sul sistema immunitario adattativo

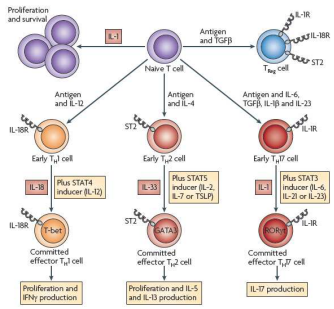


Table 2 Autoinflammatory diseases[1]

Familial Mediterranean fever
Familial cold autoinflammatory syndrome (FCAS)
Muckle-Wells syndrome
Neonatal-onset multi-inflammatory disease (NOMID)
Mevalonic aciduria
Hyper IgD syndrome
Adult-onset Still's disease
Systemic-onset juvenile idiopathic arthritis
Schnitzler's syndrome
Aim-synthetase syndrome
TNF receptor–associated periodic syndrome
Macrophage activation syndrome[2]
Behçet's syndrome
Normocomplementemic urticarial vasculitis
Pericarditis
PAPA syndrome
Blau's syndrome
Sweet's syndrome
Urate crystal arthritis (gout)
Type 2 diabetes[3]

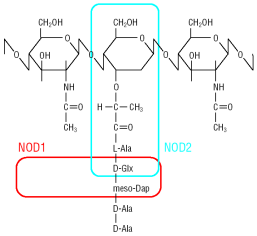
[1]Responsive primarily to IL-1 blockade.
[2]IL-18 likely contributes to the macrophage activation syndrome (20).
[3]IL-1 blockade with IL-1Ra (17) or anti-IL-1β monoclonal antibody (18) protects the insulin-producing beta cells. Hyperglycemia stimulates IL-1β secretion from the beta cell and accounts for the loss of the beta cell mass in type 2 diabetes (109).

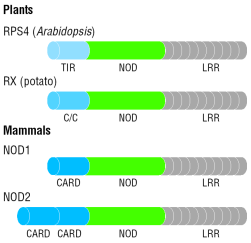
Table 3 Specific therapies for blocking IL-1 activities

Reagent	Composition	Mechanism of action	Specificity
Anakinra[a]	IL-1Ras	binds to IL-1RI>IL-1α>IL-1β	IL-1α and IL-1β
IL-1Ra trimer	IL-1Ras	binds to IL-1RI>IL-1α>IL-1β	IL-1α and IL-1β
IL-1 Trap[b]	IL-1RI + IL-1RAcP	neutralizes IL-1β>IL-1α>IL-1Ra	IL-1β (IL-1α)
Soluble IL-1RII[c]	IL-1RII	neutralizes IL-1β>IL-1α	IL-1β
Anti IL-1β	monoclonal antibody	neutralizes IL-1β	IL-1β
Anti-IL-1RI	monoclonal antibody	blocks IL-1RI	IL-1α and IL-1β
Anti-IL-1RAcP	monoclonal antibody	blocks IL-1RAcP	IL-1α and IL-1β, IL-33
Peptide antagonist[d]	RYTVFILA	blocks IL-1RI	IL-1α and IL-1β

[a]Anakinra is the recombinant (nonglycosylated) form of IL-1Ra. Naturally occurring IL-1Ra is the glycosylated gene product.
[b]Binding with Fc.
[c]Efficacy of the extracellular IL-1RII is greatly enhanced by the presence of extracellular IL-1RAcP (164, 166).
[d]Inhibits some but not all IL-1 activities (170).

NOD1 & NOD2 recognize peptidoglycan substructures and promote innate immune responses





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NOD1 and NOD2 are intracellular molecules and resemble some plant disease resistance proteins; best understood of the "NOD-like receptors" or NLRs

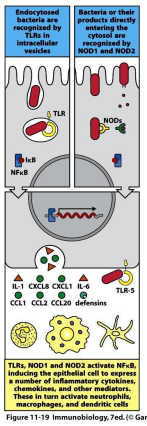


Figure 11-19 Immunobiology, 7ed. (© Garland Science 2008)

NOD signalosoma

NOD1 muramil dipeptide con acido diaminopimalico dei Gram-

NOD2 muramil dipeptide del peptidoglicano di Gram+ e Gram-

Chinasi attiva RIP2

Resistenza intestinale a *Helicobacter pylori* e *Listeria Monocytogenes*

Common alleles of NOD2 are a genetic risk factor for Crohn's disease

- Several moderately common alleles of the NOD2 gene (7% of total alleles) increase susceptibility to Crohn's disease (a form of inflammatory bowel disease)
- Two copies of these alleles increase susceptibility by 40X
- Pretty strong evidence that these alleles of are "loss of function" alleles
- NOD1/2 have been shown to have 3 immune functions: activation of inflammatory cytokine gene expression, induction of anti-microbial peptide synthesis by Paneth cells in intestines, autophagy of bacteria in cytoplasm

La famiglia della IL-12

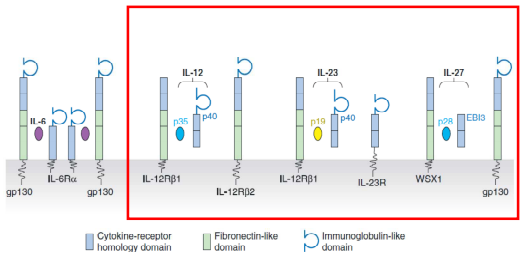
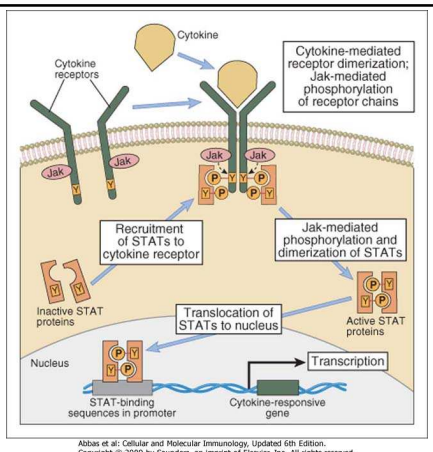


Figure 1
The IL-6/IL-12 family of cytokines.



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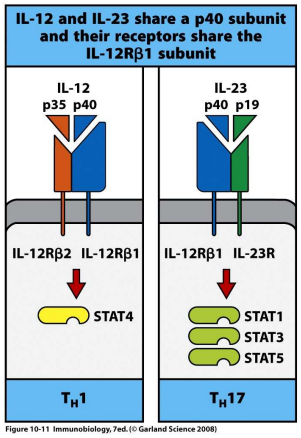


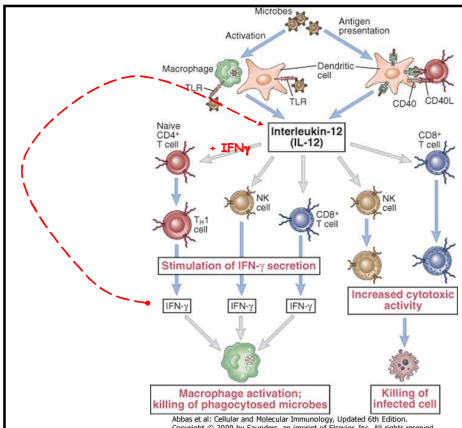
Figure 10-11 Immunobiology, 7ed. (© Garland Science 2008)

Jak/STAT	Involved cytokine(s)	Phenotype of knockout mice
STAT1	IFN-α/β, IFN-γ	Defect in innate immunity; no reponse to IFNs
STAT2	IFN-α/β	Defective immunity to viruses
STAT3	IL-6, IL-10	Embryonic lethal
STAT4	IL-12	Defect in T_H1 development, IFN-γ production
STAT5a	Prolactin	Lactation defect
STAT5b	Growth hormone (GH)	Dwarfism
STAT5a and STAT5b	IL-2, IL-7, IL-9 (also see Prolactin and GH)	Lactation defect, dwarfism, and defective T cell proliferation in response to IL-2
STAT6	IL-4	Defect in T_H2 development, and production of IL-4-dependent Ig isotypes
Jak1	IFN-α/β, IFN-γ, cytokines using γ_c and gp130 (e.g., IL-2, IL-4, IL-6)	Perinatal lethal; defective innate immunity, possible defect in neuronal viability
Jak2	Epo, IL-3, IFN-γ	Embryonic lethal, hematopoietic failure
Jak3	Cytokines using γ_c chain (IL-7, IL-2, IL-4)	Defect in T cell maturation
Tyk2	IFN-α/β, IFN-γ, IL-10, others	Defective IL-12 response of NK cells, defective immunity to viruses

Abbreviations: IFN, interferon; IL, interleukin; Jak, Janus kinase; STAT, signal transducer and activator of transcription.

Regolatori delle vie di segnalazione

- SOCS, Suppressor of cytokine signalling (competizione domini SH2 ed attivazione di ubiquitina ligasi E3)
- SHP-1 e SHP2, fostatasi tirosiniche
- PIAS, Protein Inhibitor of Activated Stats (inibitori trascrizionali)



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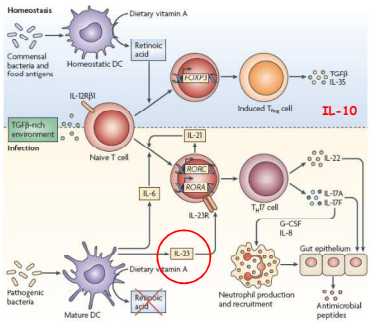
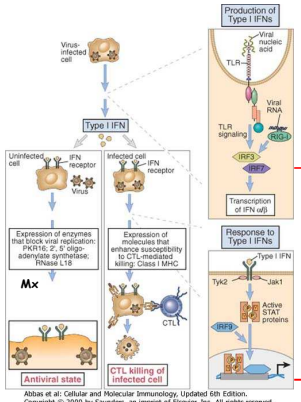
Attività biologiche di IL-23



- Patogenesi malattie autoimmuni (encefalite allergica)
- Difesa da Klebsiella

Gli interferoni di tipo I



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Double-stranded viral RNAs can be recognized by TLRs in endosomes and by RIG-I or MDA-5 in the cytosol to induce the expression of interferons

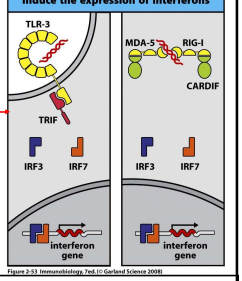
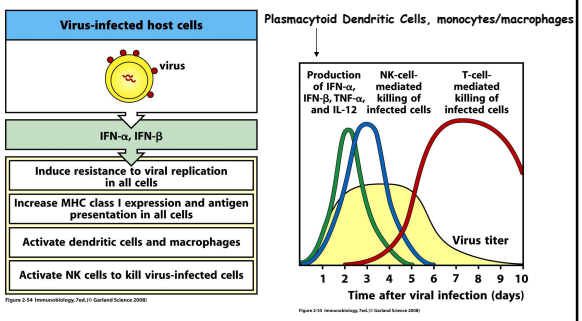


Figure 2-53 Immunobiology, 7ed. (© Garland Science 2008)

STAT1 (STAT2)

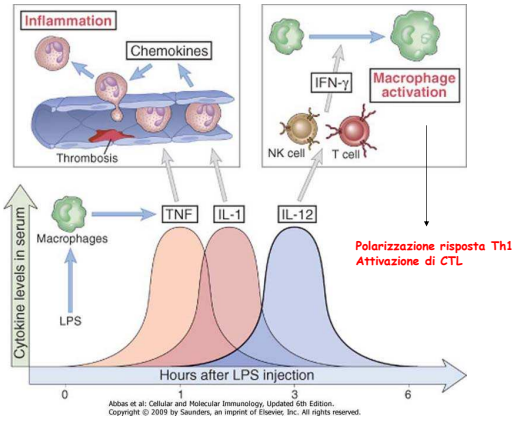
ISRE, IFN-stimulated response element
GAS, Gamma activated sites



Uso clinico prevalente degli interferoni di tipo I

IFNα: epatite e leucemia a cellule capellute

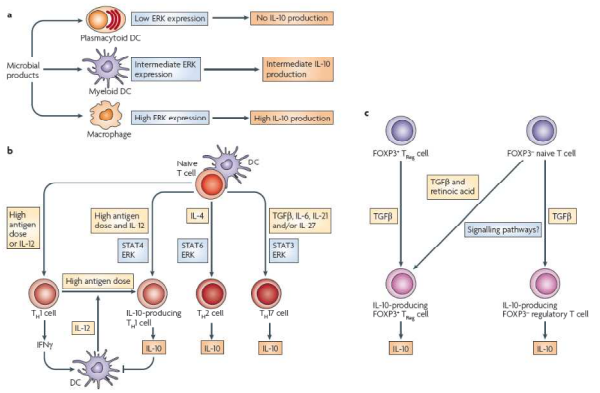
IFNβ: sclerosi multipla



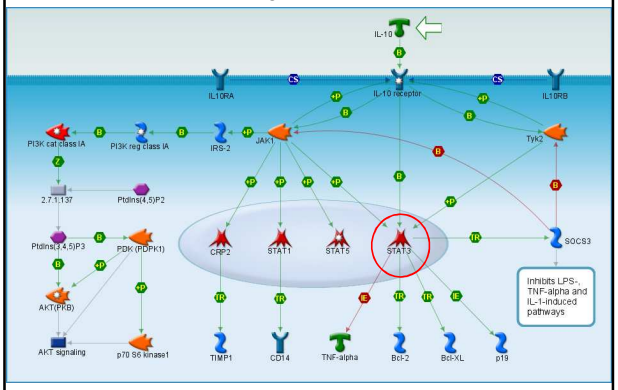
Polarizzazione risposta Th1
Attivazione di CTL

Il tipo di agente patogeno determina la popolazione cellulare prevalente nelle fasi iniziali

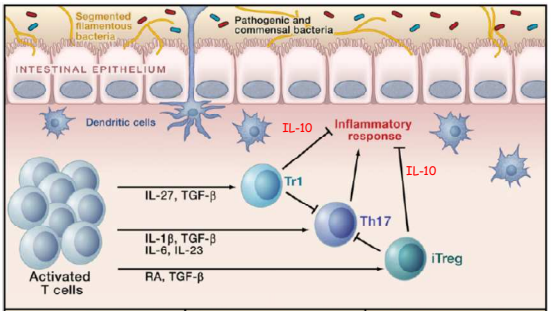
Produzione di IL-10



Vie di segnalazione di IL-10



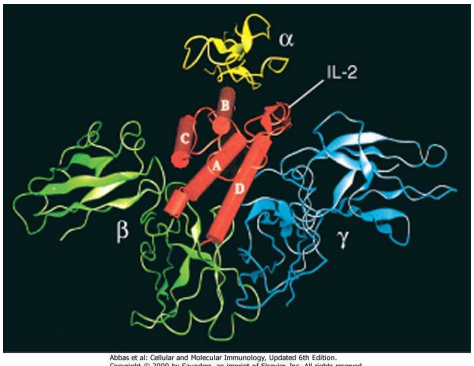
Le cellule Treg ed IL-10 spengono le risposte infiammatorie nel MALT



1. **IL-10 inibisce la produzione di IL-12 nei macrofagi e DC attivati**
2. **IL-10 inibisce espressione di molecole costimolatorie e MHC di classe II**

L'assenza di IL-10 nei topi causa malattie infiammatorie croniche dell'intestino

Interazione tra IL-2 ed il proprio recettore



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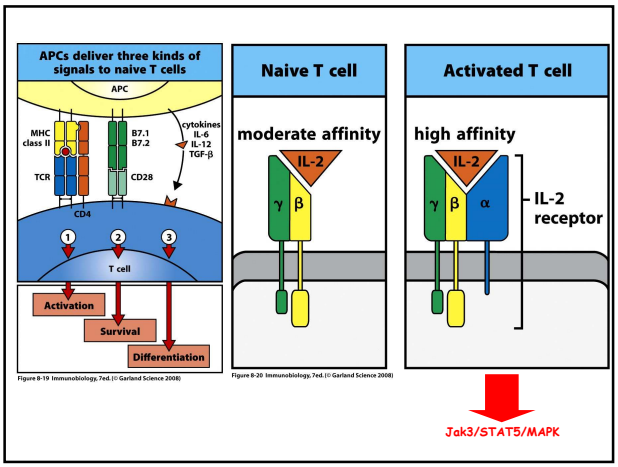
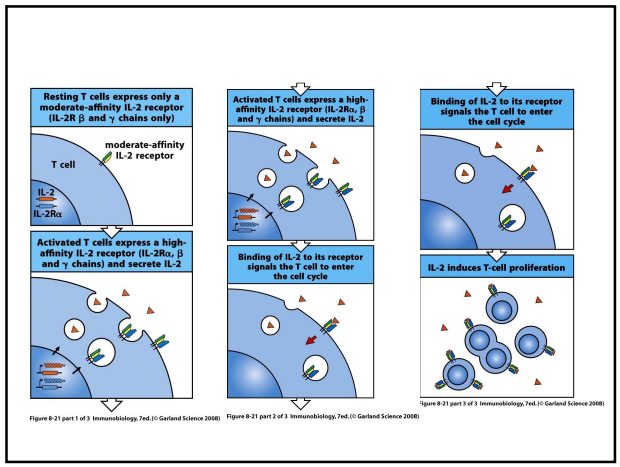
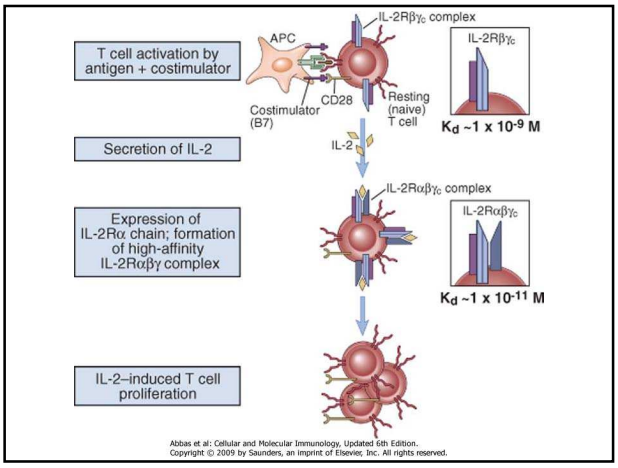
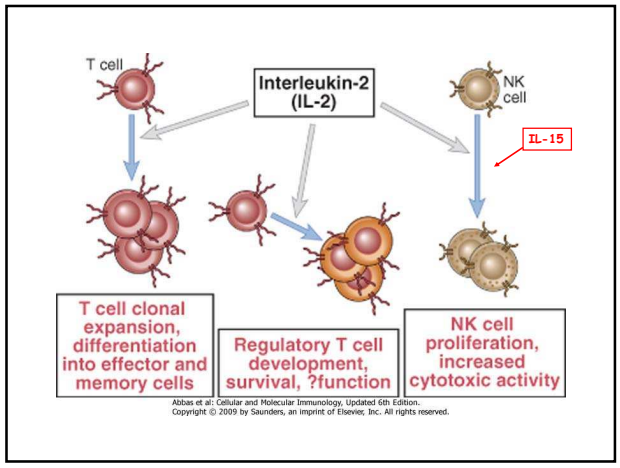
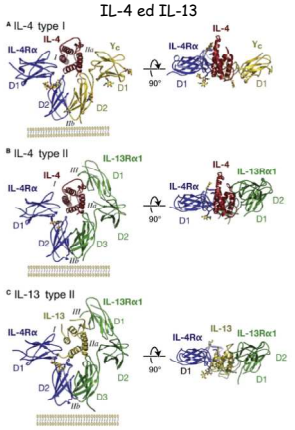




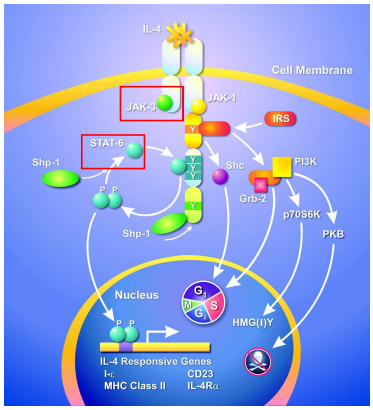




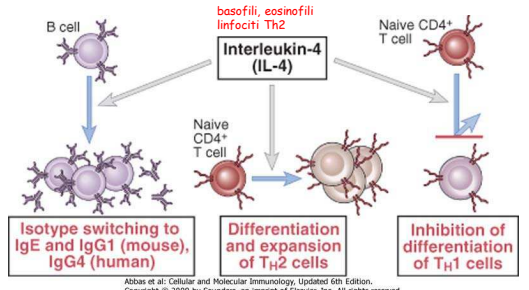
IL-4 ed IL-13



Vie di segnalazione del recettore tipo I per IL-4



Insulin Responsive Substrate



Antagonismo con IFN-γ

Azioni comuni o specifiche di IL-4 e IL-3

• Comuni: scambio isotipico delle IgE; attivazione alternativa dei macrofagi; sviluppo linfociti Th2

• Specifiche per IL-13: promozione della fibrosi durante i processi di riparazione del danno tissutale; produzione di muco da parte delle cellule caliciformi mucipare dell'epitelio bronchiale; induzione di molecole di adesione sull'endotelio (VCAM-1).

Interferone γ e l'attivazione classica dei macrofagi: segnali cellulari

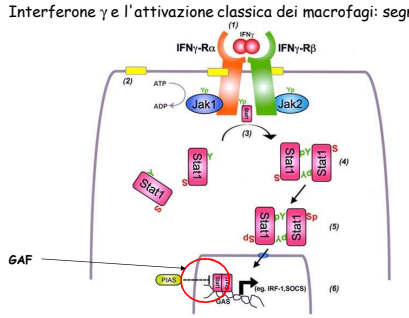
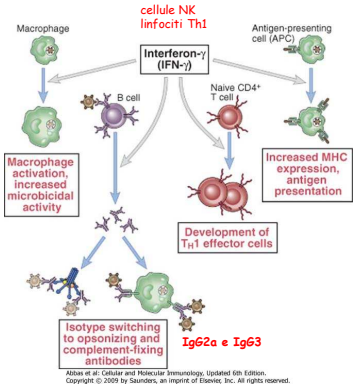
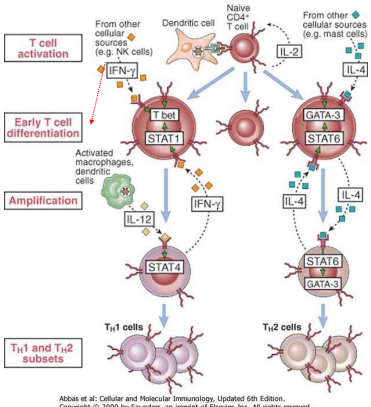


Fig. 1: schematic representation of cytokine induced Jak-Stat signal transduction using interferon-gamma as an example. Cytokine stimulation of the alpha and beta chains of the cell surface interferon gamma receptor results in autophosphorylation of both Jak 1 and Jak 2, with subsequent phosphorylation of tyrosine residues on the cytoplasmic domain of the receptor. Latent, monomeric Stat 1 normally present in the cell cytosol is then recruited to the receptor and becomes phosphorylated on tyrosine residues. Following tyrosine phosphorylation, Stat1 proteins dimerize, translocate to the nucleus, and then bind to specific sequences of DNA (GAS, the gamma activated sequence) to modulate gene transcription. Although not shown in the cartoon, Stat1 is also phosphorylated on a serine residue, which augments transcriptional activity of the activated dimer (scheme adapted from Shuai & Li 2003).

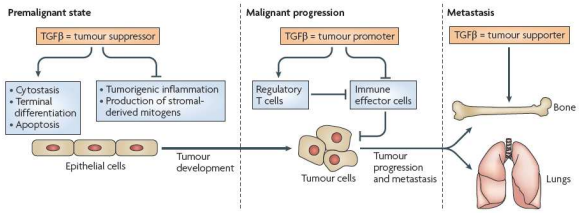
IFN-γ e l'attivazione classica dei macrofagi: funzioni



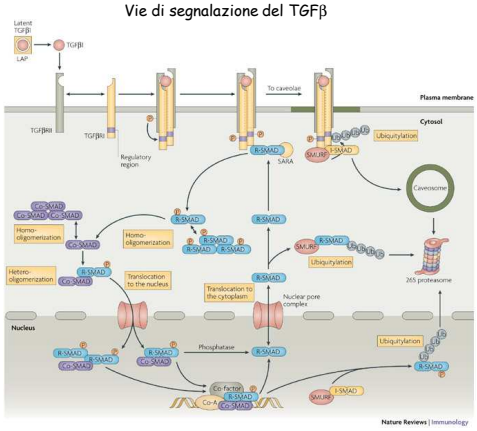
Antagonismo IFN-γ ed IL-4: differenziazione T helper



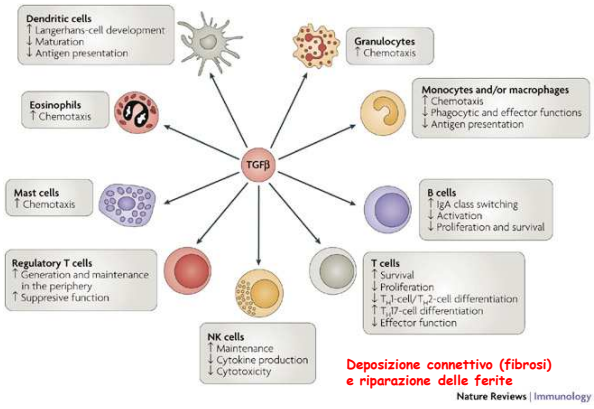
Transforming Growth Factor β (TGFβ)



Vie di segnalazione del TGFβ



Effetti biologici del TGFβ



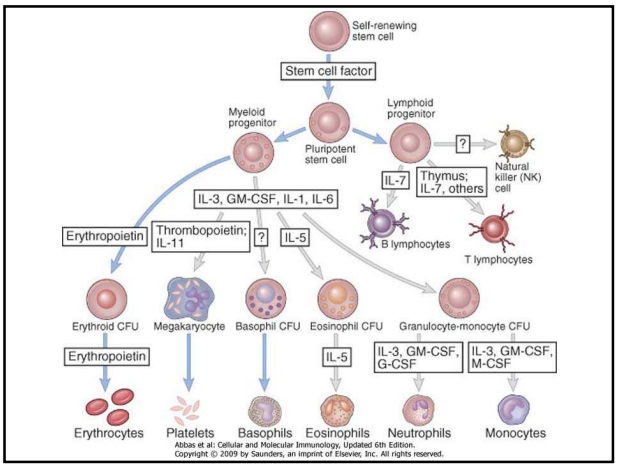


Table 12–6. Hematopoietic Cytokines

Cytokine	Size (kD)	Principal cellular sources	Principal cellular targets	Principal cell populations induced
Stem cell factor (c-Kit ligand)	24 kD	Bone marrow stromal cells	Pluripotent stem cells	All
Interleukin-7 (IL-7)	25 kD	Fibroblasts, bone marrow stromal cells	Immature lymphoid progenitors	B and T lymphocytes
Interleukin-3 (IL-3)	20–26 kD	T cells	Immature progenitors	All
Granulocyte-monocyte CSF (GM-CSF)	18–22 kD	T cells, macrophages, endothelial cells, fibroblasts	Immature and committed progenitors, mature macrophages	Granulocytes and monocytes, macrophage activity
Monocyte CSF (M-CSF)	Dimer of 70–90 kD; 40-kD subunits	Macrophages, endothelial cells, bone marrow cells, fibroblasts	Committed progenitors	Monocytes
Granulocyte CSF (G-CSF)	19 kD	Macrophages, fibroblasts, endothelial cells	Committed progenitors	Granulocytes

Abbreviations: CSF, colony-stimulating factor; kD, kilodalton.